SHORT TERM VARIABILITY IN URINARY BISPHENOL A IN AUSTRALIAN CHILDREN

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Abstract

Used frequently in food contact materials, bisphenol A (BPA) has been studied extensively in recent years, and ubiquitous exposure in the general population has been demonstrated worldwide. Characterising within- and between-individual variability of BPA concentrations is important for characterising exposure in biomonitoring studies, and this has been investigated previously in adults, but not in children. The aim of this study was to characterise the short-term variability of BPA in spot urine samples in young children. Children aged ≥2-<4 years (n = 25) were recruited from an existing cohort in Queensland Australia, and donated four spot urine samples each over a two day period. Samples were analysed for total BPA using isotope dilution online solid phase extraction-liquid chromatography-tandem mass spectrometry, and concentrations ranged from 0.53–74.5 ng/ml, with geometric mean and standard deviation of 2.70 ng/ml and 2.94 ng/ml, respectively. Sex and time of sample collection were not significant predictors of BPA concentration. The between-individual variability was approximately equal to the within-individual variability (ICC = 0.51), and this ICC is somewhat higher than previously reported literature values. This may be the result of physiological or behavioural differences between children and adults or of the relatively short exposure window assessed. Using a bootstrapping methodology, a single sample resulted in correct tertile classification approximately 70% of the time. This study suggests that single spot samples obtained from young children provide a reliable characterization of absolute and relative exposure over the short time window studied, but this may not hold true over longer timeframes.

Key words: biomonitoring; BPA; variability; intraclass correlation coefficient, children

1. Introduction

Biomonitoring provides an aggregate measure of exposure via all sources and pathways and has been hailed as the 'gold standard' for environmental exposure assessment (Sexton et al. 2004). Bisphenol A (BPA) is used as a plasticiser in polycarbonate plastics, particularly for food-contact items, and in sealers and liners for canned foods. BPA exposure was identified as being of potential concern due to evidence of estrogenic activity, and it has been studied extensively over the last decade. BPA has been linked to a range of human health outcomes including behavioural effects, disruption of endocrine function, and alteration of reproductive structure and function (reviewed in Rochester (2013)). BPA has a relatively short half-life in the range of 2-4 hours, with near-complete urinary excretion in the 24 hours following exposure (Volkel et al. 2002; Shin et al. 2004; Volkel et al. 2008; Teeguarden et al. 2011; Christensen et al. 2012b). Urinary biomonitoring is performed routinely and ubiquitous exposure has been demonstrated in non-occupationally exposed populations worldwide, including Canada and North America, Europe and the Asia-Pacific region (reviewed in Vandenberg et al. (2010)).

For BPA and many other environmental chemicals, exposure levels are often characterized based on a single spot urine sample. This can be useful in cross-sectional studies, such as the National Health and Nutrition Environmental Survey (NHANES) conducted in the United States, but has limited usefulness when trying to study a subtle endpoint, such as a potential health-outcome, or for longitudinal or long-term studies. Within- and between-individual variation in urinary concentrations is expected due to exposure differences related to diet and other lifestyle factors (Geens et al. 2012; Casas et al. 2011), and this variation is commonly what researchers aim to characterise in population-wide biomonitoring studies. However, for chemicals with short half-lives and rapid elimination kinetics such as BPA, the timing of

sample collection relative to the exposure event will also significantly influence the measured concentration (Aylward et al. 2012). This can result in exposure misclassification (Lassen et al. 2013) which has consequences for epidemiological evaluations and for risk and health assessments, so this variability should be accounted for.

A number of studies have investigated temporal variability of urinary measures of BPA. Most have focussed on adults from the general population (Arakawa et al. 2004) (Ye et al. 2011; Teitelbaum et al. 2008; Mahalingaihah et al. 2008; Nepomnaschy et al. 2009; Lassen et al. 2013; Christensen et al. 2012a) and report large within- and between-individual variation in urinary measures. Few studies have looked at variability during pregnancy (Braun et al. 2011a; Braun et al. 2012; Meeker et al. 2013; Casas et al. 2013), and none have investigated potential variability in children <6 years. Understanding exposures during critical windows of development such as childhood is particularly important because their physiology and unique susceptibility means that children are disproportionately exposed to environmental chemicals compared with adults; and early life exposures facilitate longer latency periods for the development of chronic disease in adulthood (WHO 2004, 2011; Scheuplein et al. 2002). Providing a framework for understanding biomonitoring data from children can assist in accurate risk assessment of such exposures. The aim of this study was to (1) characterise the short term variability in spot urine samples in young children; and (2) provide guidance for prospective cohort studies on the type, number, and frequency of collection for specimens for assessing short term environmental exposures to BPA in children.

2. Study population and methods

2.1 Study population

The study population consisted of 25 children aged ≥2 - <4 years (16 males, 9 females) who donated 4 urine samples over a 2 day period. The participants were recruited from an ongoing study of iodine status and thyroid function in South-East Queensland, Australia. Descriptive information about each specimen was limited to age, postal code and sex. Care-givers of participants were instructed to collect one morning and one afternoon spot sample (first morning void and first urine following the midday meal, respectively) on two consecutive days, to give a total of 4 samples per participant. Samples were collected from toilet-trained children in standard polyethylene urine specimen containers and frozen until analysis. Specimens were collected from May 2012 to March 2013, and analysed in April-May 2013. No measures of creatinine or specific gravity were available. This study was approved by the University of Queensland ethics committee (approval number 2011000125).

2.2 Chemical analysis

Urine samples were analysed for total BPA (free plus conjugated species) at the National Research Centre for Environmental Toxicology, University of Queensland, Australia using an online solid-phase extraction liquid chromatography tandem mass spectrometry method. Briefly, 50 µL urine was diluted, cleaved enzymatically and injected directly into the online system using a GX-271 liquid handler. Quantitation was performed by isotope dilution using ^{13}C-BPA (Cambridge Isotope Laboratories). More details can be found in Heffernan et al. (2013). Synthetic urine (Calafat and Sampson 2009) was used for quality control. Fortified synthetic urine (1 ng/ml) was used to monitor instrument performance (0.91 ± 0.24 ng/ml, n = 13), and background contamination was monitored by repeated measures of un-fortified synthetic urine (0.18 ± 0.021 ng/ml, n = 11).The limit of detection (LOD) was 0.062 ng/ml (calculated as 3*standard deviation of the blank). As blank levels were significantly higher

than the LOD, the limit of reporting (LOR) was calculated as 3*average blank, and set as 0.53 ng/ml. No blank subtraction was performed.

2.3 Exposure estimation

Using model-predicted, age specific urinary flow, F (ml/kg d^{-1}) constructed from published literature values (Ballauf et al. 1988, Ebner and Manz 2002, Goellner et al. 1981, Magos 1987, Maguire et al. 2007, Martins et al. 2011, Pratt et al. 1948, Roberts and Lucas 1985) described previously (Heffernan et al. 2013), and measured pool concentrations, C (ng/ml), daily urinary excretion of BPA, E (ng/kg-d), was calculated for each pool according to the following:

$$E = F * C \qquad \text{(equation 1)}$$

Under a steady-state assumption, daily urinary excretion of BPA will be equal to daily intake (Volkel et al. 2002); therefore estimated daily BPA excretion can be taken as estimates of daily BPA intake in the population covered by this study. This approach explicitly assumes that the BPA urinary excretion rate during the time period covered by the urinary void sampled is consistent over a 24-hour period, and the flow rate, excretion and exposure data are presented as estimates only, and for comparison to guideline intake values.

2.4 Statistical analysis

Intraclass correlation coefficients (ICC) were calculated using Stata IC12 (Stata Corp., College Station, TX, USA). A mixed-effects model was implemented to assess the within- and between-individual variance, and ICC values were reported as the ratio of the between-individual variance to the total variance.

Using a bootstrapping exercise, the dataset of four samples per individual over a two-day period was used to assess the relative reduction in accuracy in exposure classification resulting from collection of fewer than 4 samples over the same period. The dataset was resampled to simulate collection of 1, 2, 3, or 4 samples from each individual. For each iteration a new geometric mean (GM) value was calculated for each individual based on the resampled simulated dataset. The Spearman rank correlation coefficient (rho, ρ) between the simulated GM and the 'true' or observed GM (from the original dataset) was calculated for each participant for each iteration. In addition, each individual was categorized into tertile by simulated GM, and the resulting categorization was compared to that based on the 'true' GM; the fraction that was correctly categorized was recorded. The simulation was conducted for 1,000 iterations for each number of spot samples per individual (1 through 4). The resulting median Spearman rank correlation coefficients and the fraction of participants correctly categorized from the 1,000 iterations are reported, along with confidence intervals estimated at the 2.5^{th} and 97.5^{th} percentiles of the results from the 1,000 iterations (Table 2)

3. Results

BPA was detected in all samples, with 5% (n = 5) being <LOR. Concentrations ranged from <LOR – 74.5 ng/ml with a GM of 2.70 and geometric standard deviation (GSD) of 2.94 ng/ml (Table 1). One individual (participant nine) was clearly an outlier. Of the four samples from this individual, three were the highest in the dataset and the fourth sample from that individual was the sixth highest in the dataset. If that individual is omitted from the dataset, the GM and GSD are 2.45 and 2.64 ng/ml.

A multivariate regression model was used to assess the influence of sex and time of sample collection (classified as morning or afternoon) on BPA concentration. Neither variable was found to be a significant predictor of measured BPA concentrations. Between-individual variance for the repeat sampling was approximately equal to within-individual variance (ICC = 0.51; 95% CI 0.32-0.70). To test the sensitivity of the assessment, the individual with the most extreme values (participant nine) was omitted and the calculation repeated. The ICC decreased slightly to 0.40 (95% CI 0.21-0.62). In either case, this represents moderate within-person variability, suggesting that a single sample will provide a moderately reliable assessment of relative exposure levels amongst participants.

The relative accuracy of using 1, 2, 3 or 4 samples to classify participants with respect to exposure over the collection time frame (48 hours) was assessed via bootstrapping. The resulting median Spearman rank correlation coefficients between the bootstrapped datasets and the 'true' 4-sample mean ranged from 0.81 to 0.93 based on the use of 1 to 4 samples. Often the results of biomonitoring studies are interpreted categorically for epidemiological study, with outcomes assessed in subjects in upper categories compared to those observed in the lowest quantile. Therefore, an assessment of the accuracy of exposure classification based on the number of samples used may be of interest to researchers. Using the same bootstrapping methodology, each individual was categorised by tertile and compared to the 'true' mean. The fraction of individuals correctly categorised based on 1, 2, 3 or 4 samples is presented in Table 2. If one sample is used to categorise exposure an estimated 68% of individuals would be correctly classified, compared with 84% of individuals if four samples were used.

4. Discussion

The study population was ubiquitously exposed to BPA, and although biomonitoring data for children <5 years is sparse, the range (0.53–74.5 ng/ml) and GM (2.70 ng/ml) are consistent with the literature values available. Braun et al. (2011b) reported median concentrations of 2.9 ng/ml for 2 year old (n = 195) and 3 year old (n = 222) children in the United States and similar results were reported in other smaller studies by (Casas et al. 2011, Morgan et al. 2011, Pirard et al. 2012). A single participant had significantly higher urine concentrations for all four samples than any other individual in the study (participant nine, Figure 1). Replicate samples from this participant were re-extracted and reanalysed and produced comparable results, and method blanks were low and consistent, indicating little or no contamination of the sample in the laboratory during storage or analysis. As no sample collection instructions specifically aimed at reducing potential contamination were given to participants, contamination of the samples with the free species during sample collection was possible. To assess this samples >95th% (n = 5) were reanalysed separately for free and total BPA. Free BPA was >LOR in a single sample, and accounted for 3% of total BPA. This confirms that quality control procedures were adequate to prevent and assess potential contamination with the free species. The results for participant nine (14.6 - 74.5 ng/ml) are consistent with the range of concentrations measured by Teeguarden et al. (2011) (1.21-125 ng/ml) in a study of individuals who had consumed a BPA-rich diet, suggesting that the consistently high urinary concentrations for participant 9 may be the result of consumption of relatively highly-contaminated foodstuffs by this individual during the study period.

Using methods described by Caudill (2010, 2012) values for a bias-corrected GM, GSD, and an upper-bound reference value (95th percentile, (95th%)) were calculated from an existing data set which utilised pooled spot urine samples (n = 30 pools, where 7 individuals contributed to each pool) from Australian children of the same age (≥2 - <4 years) (Heffernan

et al. 2013). Using the calculated GM and GSD values based on the pooled sampling effort (1.20 and 3.54 ng/ml, respectively) a population reference value at the 95th percentile was estimated as 9.59 ng/ml. This level is denoted by a dashed line in Figure 1 for comparison to data from the current study. In the current study 10% and 6.3% of samples (including and excluding participant nine, respectively) exceeded this upper-bound reference value, suggesting that application of the Caudill (2010, 2012) methods to the pooled samples from the previous study does provide a useful characterisation of the general distribution of values in the underlying population.

A urine flow model described previously (Heffernan et al. 2013) was applied to account for age related differences in daily urinary output (Aperia et al. 1984) and to estimate BPA exposure, assuming that the urinary excretion rate of BPA during the time period covered by the urinary void sampled is consistent over a 24-hour period. The GM exposure estimate for the population is 86.5 ng/kg d^{-1} (Table 2) which is consistent with a previous exposure estimate for Australian children (88.3 ng/kg d^{-1}, n = 30 pools of 7, 2-4 years) (Heffernan et al. 2013). The current TDI set by European Food Safety Authority (EFSA) is 50 µg/kg d^{-1} (EFSA 2010), although some researchers (e.g. Willhite et al. (2008)) have suggested adoption of a more conservative value. For the highest concentration samples (from participant nine) the excretion estimate based on a single spot sample (the most concentrated) is 3190 ng/kg d^{-1}, but decreases by more than 50% to 1540 ng/kg d^{-1} if the four sample mean is used. This estimate is more than 15-fold lower than the EFSA guideline, and is five-fold lower than the 16 µg/kg d^{-1} suggested by Willhite et al. (2008).

4.1 Within- and between-individual variation

The ICC was calculated to assess the magnitude of the within- and between-person variability. The calculation is sensitive to extreme values (note change in the ICC from 0.51 to 0.40 with the exclusion of participant nine), but the ICC in this study is somewhat higher than previously reported literature values for urinary BPA, with a typical range of 0.1-0.4 (recently reviewed in Aylward et al. (2012)). In a recent study Lassen et al. (2013) compared spot, first morning and 24-hour urine samples over a 3 month period, and the ICC values based on unadjusted urinary concentrations were 0.42, 0.1 and 0.26, respectively. The ICC based on two spot samples collected four days apart was similar to that calculated in the current study, but the measurement of first morning voids or 24-hour samples over a longer period of time suggests significantly greater within-person variability as longer time periods are considered.

The relatively high ICC in the current study compared to previous studies may be explained by the fact that the majority of the previously published ICC values have been based on sampling in adults, and the results from adults and children, especially very young children, may demonstrate different patterns. Mouthing behaviours in children contribute to non-nutritive ingestion (Tulve et al. 2002; Xue et al. 2007), which may increase the potential for buccal absorption, which bypasses first-pass hepatic metabolism of BPA, and may alter the serum-urine time metabolic profile (Gayrard et al. 2013). Assuming diet is the predominant exposure pathway for BPA (Geens et al. 2012), it is possible that children have a different exposure profile due to different patterns of food consumption, with smaller but more frequent meals. This could potentially result in lower within-individual, within-day variation in BPA exposure and excretion rates. Similarly, because children often have more homogeneous diets than adults (Huybrechts et al. 2008; Nelson et al. 1989), and this effect

may be more pronounced in very young children, one might expect between-day variation to be lower in infants compared to children, and lower in both of these compared to adults.

One of the limitations of the study is that a relatively short exposure window was assessed. It is possible that the number of samples collected, the length of the study, or both, was insufficient to allow accurate characterization of the within-person variability exhibited over a longer time period. Previous studies used samples collected at various time points over several days (Ye et al. 2011), weeks (Nepomnaschy et al. 2009), or months (Mahalingaihah et al. 2008; Lassen et al. 2013). If samples in the current study were collected over a longer time period, it is possible that within-person variability would increase relative to total variation, resulting in a lower ICC similar to previous studies.

4.2 Exposure classification

The bootstrapping analysis of the data collected in this study suggested that over a short time period, the use of a single sample resulted in the correct classification of participants by tertile approximately 70 percent of the time. In a study of black and Hispanic children in the U.S (6-10 years) Teitelbaum et al. (2008) contended that a single spot sample could be used to rank study participants by exposure level (based on the 6-month average determined from 6 spot samples collected approximately 1 month apart) based on ICC values of 0.22 (0.35 using creatinine-corrected concentrations). Similarly, Mahalingaihah et al. (2008) used samples collected from women attending a fertility clinic to examine the predicative power of a single spot sample to correctly classify exposure in the highest tertile based on a three- or six-sample mean. The predictive power of a single sample over the course of 6 months (mean 172 days) was 0.63, and increased to 0.84 using two samples over 10 months (mean 304 days).

Evidence from this and other studies suggests that ubiquitous and daily exposure to BPA results in a relative pseudo-steady state over months or years (National Research Council 2006). However, specific conclusions on exposure level based on urinary biomonitoring for BPA must consider the purpose of the assessment. If estimating intake levels is the goal, the within-day, within-person variation in urinary concentrations due to the peaks and troughs expected due to rapid absorption and urinary elimination rates should be considered before relying on extremes in the population distribution of spot sample concentrations to characterise intake rates (Christensen et al. 2012a; Ye et al. 2011; Aylward et al. 2012). If categorization of small children with respect to relative BPA exposure levels is the goal, single spot samples may be useful, but potential changes or variability in exposure levels over time among individuals should be considered. Due to the low ICCs for BPA observed in other studies, decisions regarding sampling strategy should consider the goals of the study. If short-term exposure characterization is the goal, a single spot sample may be appropriate. However, if characterization of chronic exposure patters is a goal, for example, in studies of health outcomes potentially associated with chronic exposure levels, repeated sampling may be required. The data presented here add to the sparse literature on urinary BPA concentrations in children ages 2 to 4 years and suggest that, at least over a limited time period, single spot samples may provide relatively robust characterization of absolute and relative exposure levels in young children.

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Tables

Table 1: Urinary BPA concentrations and estimated excretion in 2 – 3 year old children

			Concentration (ng/ml)						GM estimated excretion ($ng/kg\ d^{-1}$)
	n	# > LOR (%)	Range	GM	25%	50%	75%	95%	
Males	64	60/64 (94%)	<0.53 – 74.5	3.09	1.74	3.22	6.26	17.2	99.1
Females	36	35/36 (97%)	<0.53 – 16.1	2.17	1.16	2.27	3.55	10.3	68.5
Total	100	95/100 (95%)	<0.53 – 74.5	2.72	1.57	2.74	4.68	14.6	86.5

Table 2: Results for Spearman's rank correlation coefficient (ρ) between generated mean and observed 4-sample mean (average of 1000 iterations); and fraction correctly ranked by tertile based on 'true' 4-sample mean; 95% CI = 95% confidence interval

Number of repeated samples	ρ to observed 4-sample mean (95% CI)	Fraction of subjects ranked in correct tertile based on observed 4-sample mean (95% CI)
1	0.81 (0.64-0.91)	0.68 (0.52-0.84)
2	0.88 (0.77-0.94)	0.76 (0.60-0.92)
3	0.91 (0.83-0.96)	0.80 (0.68-0.92)
4	0.93 (0.86-0.97)	0.84 (0.68-0.92)

Figures

Figure 1: Within- and between person variation in urinary total BPA concentrations in 2-4 year old children. Horizontal bars denote 2-day arithmetic mean urinary concentration. Dashed line indicates estimated population 95th percentile for ages 2-4 years calculated based on data from a previous pooled study (see Heffernan et al 2013); dot-dashed line indicates method limit of reporting